THE CLAIMS

What is Claimed Is:

1. A method of determining the presence or absence of a target substance in a test sample, comprising:

a) providing an electrode comprising a conductive substrate modified with a non-conductive layer having an immobilized first binder capable of binding the target substance and through which layer a transition metal mediator can freely move to transfer electrons to the conductive substrate;

b) contacting the immobilized first binder with the test sample to form a target complex if the target substance is present in the test sample;

c) contacting the first binder or the target complex, if present, with a second binder capable of binding the target substance and having an endogenous or exogenous label capable of being oxidized in an oxidation-reduction reaction;

d) contacting the electrode, the immobilized first binder, and the target complex having the second binder, if present, with a transition metal mediator that oxidizes the label in an oxidation-reduction reaction between the transition metal mediator and the label, from which label there is electron transfer to the transition metal mediator resulting in regeneration of the reduced form of the transition metal mediator as part of a catalytic cycle;

e) detecting the oxidation-reduction reaction; and

f) determining the presence or absence of the target substance in the test sample from the detected oxidation-reduction reaction.

2. The method of claim 1, wherein the target substance is selected from the group consisting of proteins, protein fragments, ligands, carbohydrates, drugs, drug candidates and hormones.

3. The method of claim 1, wherein the immobilized first binder is selected from the group consisting of immunoglobulins, receptors, proteins, and oligonucleotides.

4. The method of claim 3, wherein the immobilized first binder is a receptor of eukaryotic, prokaryotic or viral origin.

5. The method of claim 3, wherein the immobilized first binder is an extracellular matrix protein.

6. The method of claim 1, wherein the second binder is labeled with an exogenous label.

7. The method of claim 6, wherein the label is an oligonucleotide.

8. The method of claim 1 or 6, wherein the label is a peptide containing amino acids capable of being oxidized in an oxidation-reduction reaction.

9. The method of claim 8, wherein the peptide label contains one or more amino acids capable of being oxidized in an oxidation-reduction reaction at approximately $\leq 0.6V$.

10. The method of claim 9, wherein the transition metal mediator is osmium^{2+}(4,4'-dimethyl-2,2'-bipyridine)$_3$.

11. The method of claim 1, wherein the test sample and the second binder are added to the immobilized first binder simultaneously.

12. The method of claim 1, wherein the nonconductive layer is the immobilized first binder.

13. The method of claim 1, wherein the nonconductive layer to which the first binder is immobilized is selected from the group consisting of streptavidin, avidin, protein A, protein G, and antibodies.

14. The method of claim 1, wherein the nonconductive layer to which the first binder is immobilized is a silane molecule covalently attached to the conductive substrate, said silane molecule further being capable of forming a covalent bond with the first binder.

15. The method of claim 1, wherein the nonconductive layer to which the first binder is immobilized comprises one or more components.

16. The method of claim 2, wherein the target substance is a protein.

17. The method of claim 16, wherein the immobilized first binder is selected from the group consisting of immunoglobulins, receptors, proteins, and oligonucleotides.

18. The method of claim 17, wherein the immobilized first binder is a receptor of eukaryotic, prokaryotic or viral origin.

19. The method of claim 17, wherein the immobilized first binder is an extracellular matrix protein.

20. The method of claim 16, wherein the second binder is labeled with an exogenous label.

21. The method of claim 20, wherein the label is an oligonucleotide.

22. The method of claim 20, wherein the label is a peptide containing amino acids capable of being oxidized in an oxidation-reduction reaction.

23. The method of claim 22, wherein the peptide label contains one or more amino acids capable of being oxidized in an oxidation-reduction reaction at approximately ≤ 0.6V.

24. The method of claim 23, wherein the transition metal mediator is osmium^{2+}(4,4'-dimethyl-2,2'-bipyridine)$_3$.

25. The method of claim 16, wherein the test sample and the second binder are added to the immobilized first binder simultaneously.

26. The method of claim 16, wherein the nonconductive layer is the immobilized first binder.

27. The method of claim 16, wherein the nonconductive layer to which the first binder is immobilized is selected from the group consisting of streptavidin, avidin, protein A, protein G, and antibodies.

28. The method of claim 16, wherein the nonconductive layer to which the first binder is immobilized is a silane molecule covalently attached to the conductive substrate, said silane molecule further capable of forming a covalent bond with the first binder.

29. The method of claim 16, wherein the nonconductive layer to which the first binder is immobilized comprises one or more components.

30. A method of determining the presence or absence of a target substance in a test sample, comprising:

a) providing an electrode comprising a conductive substrate modified with a non-conductive layer having an immobilized binder capable of binding the target substance and through which layer a transition metal mediator can freely move to transfer electrons to the conductive substrate;

b) contacting the immobilized binder with the test sample to form a target complex if the target substance is present in the test sample;

c) contacting the immobilized binder with an endogenously or exogenously labeled substance capable of binding with the immobilized binder, such that binding of the labeled substance is inhibited if the target complex is present, and wherein the label is capable of being oxidized in an oxidation-reduction reaction;

d) contacting the electrode, the immobilized binder, the target substance, and the labeled substance, if present, with a transition metal mediator that oxidizes the label in an oxidation-reduction reaction between the transition metal mediator and the label, from which label there is electron transfer to the transition metal mediator resulting in regeneration of the reduced form of the transition metal mediator as part of a catalytic cycle;

e) detecting the oxidation-reduction reaction; and

f) determining the presence or absence of the target substance in the test sample from the detected oxidation-reduction reaction.

31. The method of claim 30, wherein the target substance is selected from the group consisting of proteins, protein fragments, ligands, carbohydrates, drugs, drug candidates, steroids and hormones.

32. The method of claim 30, wherein the test sample and labeled substance are added to the immobilized binder simultaneously.

33. The method of claim 30, wherein the immobilized binder is selected from the group consisting of immunoglobulins, receptors, proteins, and oligonucleotides.

34. The method of claim 33, wherein the immobilized binder is a receptor of eukaryotic, prokaryotic or viral origin.

35. The method of claim 33, wherein the immobilized binder is an extracellular matrix protein.

36. The method of claim 30, wherein the label is an exogenous label.

37. The method of claim 36, wherein the label is an oligonucleotide.

38. The method of claim 36, wherein the label is a peptide containing amino acids capable of being oxidized in an oxidation-reduction reaction.

39. The method of claim 38, wherein the peptide label contains one or more amino acids capable of being oxidized in an oxidation-reduction reaction at approximately $\leq 0.6V$.

40. The method of claim 39, wherein the transition metal mediator is osmium^{2+}(4,4'-dimethyl-2,2'-bipyridine)$_3$.

41. The method of claim 30, wherein the labeled substance is selected from the group consisting of proteins, protein fragments, recombinant proteins

and recombinant protein fragments, ligands, carbohydrates, drugs, drug candidates, steroids and hormones.

42. The method of claim 30, wherein the nonconductive layer is the immobilized binder.

43. The method of claim 30, wherein the nonconductive layer to which the binder is immobilized is selected from the group consisting of streptavidin, avidin, protein A, protein G, and antibodies.

44. The method of claim 30, wherein the nonconductive layer to which the binder is immobilized is a silane molecule covalently attached to the conductive substrate, said silane molecule further capable of forming a covalent bond with the binder.

45. The method of claim 30, wherein the nonconductive layer to which the binder is immobilized comprises one or more components.

46. The method of claim 31, wherein the target substance is a protein.

47. The method of claim 46, wherein the test sample and the labeled substance are added to the immobilized binder simultaneously.

48. The method of claim 46, wherein the immobilized binder is selected from the group consisting of immunoglobulins, receptors, proteins, and oligonucleotides.

49. The method of claim 48, wherein the immobilized binder is a receptor of eukaryotic, prokaryotic or viral origin.

50. The method of claim 48, wherein the immobilized first binder is an extracellular matrix protein.

51. The method of claim 46, wherein the label is an exogenous label.

52. The method of claim 51, wherein the label is an oligonucleotide.

53. The method of claim 51, wherein the label is a peptide containing amino acids capable of being oxidized in an oxidation-reduction reaction.

54. The method of claim 53, wherein the peptide label contains one or more amino acids capable of being oxidized in an oxidation-reduction reaction at approximately $\leq 0.6V$.

55. The method of claim 54, wherein the transition metal mediator is osmium^{2+}(4,4'-dimethyl-2,2'-bipyridine)$_3$.

56. The method of claim 46, wherein the labeled substance is selected from the group consisting of proteins and recombinant proteins.

57. The method of claim 46, wherein the nonconductive layer is the immobilized binder.

58. The method of claim 46, wherein the nonconductive layer to which the binder is immobilized is selected from the group consisting of streptavidin, avidin, protein A, protein G, and antibodies.

59. The method of claim 46, wherein the nonconductive layer to which the binder is immobilized is a silane molecule covalently attached to the

conductive substrate, said silane molecule further capable of forming a covalent bond with the binder.

60. The method of claim 46, wherein the nonconductive layer to which the binder is immobilized comprises one or more components.

61. A method of determining the presence or absence of a target substance in a test sample, comprising:

a) providing an electrode comprising a conductive substrate modified with a non-conductive layer having an immobilized binder capable of binding the target substance and through which layer a transition metal mediator can freely move to transfer electrons to the conductive substrate;

b) contacting the immobilized binder with a surrogate target capable of binding with the immobilized binder to form a target complex, said surrogate target having an endogenous or exogenous label capable of being oxidized in an oxidation-reduction reaction;

c) contacting the target complex with the test sample, so that labeled surrogate target is displaced from the immobilized binder by the target substance, if the target substance is present in the test sample;

d) contacting the electrode, the immobilized binder, and said surrogate target, if present, with a transition metal mediator that oxidizes the label in an oxidation-reduction reaction between the transition metal mediator and the label, from which label there is electron transfer to the transition metal mediator resulting in regeneration of the reduced form of the transition metal mediator as part of a catalytic cycle;

e) detecting the oxidation-reduction reaction; and

f) determining the presence or absence of the target substance in the test sample from the detected oxidation-reduction reaction.

62. The method of claim 61, wherein the target substance is selected from the group consisting of proteins, protein fragments, ligands, carbohydrates, drugs, drug candidates, steroids and hormones.

63. The method of claim 61, wherein the immobilized binder is selected from the group consisting of immunoglobulins, receptors, proteins, and oligonucleotides.

64. The method of claim 63, wherein the immobilized binder is a receptor of eukaryotic, prokaryotic or viral origin.

65. The method of claim 63, wherein the immobilized binder is an extracellular matrix protein.

66. The method of claim 61, wherein the label is an exogenous label.

67. The method of claim 66, wherein with the label is an oligonucleotide.

68. The method of claim 66, wherein the label is a peptide containing amino acids capable of being oxidized in an oxidation-reduction reaction.

69. The method of claim 68, wherein the peptide label contains one or more amino acids capable of being oxidized in an oxidation-reduction reaction at approximately $\leq 0.6V$.

70. The method of claim 69, wherein the transition metal mediator is osmium^{2+}(4,4'-dimethyl-2,2'-bipyridine)$_3$.

71. The method of claim 61, wherein the labeled surrogate target is selected from the group consisting of proteins, protein fragments, recombinant

proteins and recombinant protein fragments, ligands, carbohydrates, drugs, drug candidates, steroids and hormones.

72. The method of claim 61, wherein the nonconductive layer is the immobilized binder.

73. The method of claim 61, wherein the nonconductive layer to which the binder is immobilized is selected from the group consisting of streptavidin, avidin, protein A, protein G, and antibodies.

74. The method of claim 61, wherein the nonconductive layer to which the binder is immobilized is a silane molecule covalently attached to the conductive substrate, said silane molecule further capable of forming a covalent bond with the binder.

75. The method of claim 61, wherein the nonconductive layer to which the binder is immobilized comprises one or more components.

76. A method of determining the presence or absence of a target substance in a test sample comprising:

a) providing an electrode comprising a conductive substrate modified with a non-conductive layer having an immobilized target substance or an immobilized surrogate target substance, and through which layer a transition metal mediator can freely move to transfer electrons to the conductive substrate;

b) contacting the immobilized target substance or immobilized surrogate target substance with the test sample and with an endogenously or exogenously labeled binder that will bind the target substance in the test sample such that the target substance in the test sample, if present, competes with the immobilized target substance or the immobilized

surrogate target substance for the labeled binder, said label being capable of being oxidized in an oxidation-reduction reaction;

c) contacting the electrode, the immobilized target substance or immobilized surrogate target substance, and the labeled binder, if present, with a transition metal mediator that oxidizes the label in an oxidation-reduction reaction between the transition metal mediator and the label, from which label there is electron transfer to the transition metal mediator resulting in regeneration of the reduced form of the transition metal mediator as part of a catalytic cycle;

d) detecting the oxidation-reduction reaction; and

e) determining the presence or absence of target substance in the test sample from the detected oxidation-reduction reaction.

77. The method of claim 76, wherein the target substance is selected from the group consisting of proteins, protein fragments, ligands, carbohydrates, drugs, drug candidates, steroids and hormones.

78. The method of claim 76, wherein the labeled binder and the test sample are mixed prior to being added to the immobilized target substance or immobilized surrogate target substance.

79. The method of claim 76, wherein the nonconductive layer is the immobilized target substance or immobilized surrogate target substance.

80. The method of claim 76, wherein the immobilized target substance or immobilized surrogate target substance is selected from the group consisting of proteins and recombinant proteins.

81. The method of claim 76, wherein the labeled binder is selected from the group consisting of immunoglobulins, receptors, proteins, and oligonucleotides.

82. The method of claim 81, wherein the labeled binder is a receptor of eukaryotic, prokaryotic or viral origin.

83. The method of claim 81, wherein the labeled binder is an extracellular matrix protein.

84. The method of claim 81, wherein the label is an exogenous label.

85. The method of claim 84, wherein with the label is an oligonucleotide.

86. The method of claim 84, wherein the label is a peptide containing amino acids capable of being oxidized in an oxidation-reduction reaction.

87. The method of claim 86, wherein the peptide label contains one or more amino acids capable of being oxidized in an oxidation-reduction reaction at approximately $\leq 0.6V$.

88. The method of claim 87, wherein the transition metal mediator is osmium^{2+}(4,4'-dimethyl-2,2'-bipyridine)$_3$.

89. The method of claim 76, wherein the nonconductive layer to which the immobilized target substance or immobilized surrogate target substance is immobilized is selected from the group consisting of streptavidin, or avidin, protein A, protein G and antibodies.

90. The method of claim 76, wherein the nonconductive layer to which the immobilized target substance or immobilized surrogate target substance is immobilized is a silane molecule covalently attached to the conductive substrate, said silane molecule further capable of forming a covalent bond with the immobilized target substance or immobilized surrogate target substance.

91. The method of claim 76, wherein the nonconductive layer comprises one or more components.

92. A method of determining the effect of a test sample on the binding interactions between two binders that are members of a binding pair, said method comprising:

a) providing an electrode comprising a conductive substrate modified with a non-conductive layer having an immobilized first binder and through which layer a transition metal mediator can freely move to transfer electrons to the conductive substrate;

b) contacting the immobilized first binder with the test sample;

c) contacting the immobilized first binder with an endogenously or exogenously labeled second binder for said first binder, said label being capable of being oxidized in an oxidation-reduction reaction;

d) contacting the electrode, the immobilized first binder, and the labeled second binder, if present, with a transition metal mediator that oxidizes the label in an oxidation-reduction reaction between the transition metal mediator and the label, from which label there is electron transfer to the transition metal mediator resulting in regeneration of the reduced form of the transition metal mediator as part of a catalytic cycle;

e) detecting the oxidation-reduction reaction; and

f) determining the effect of the test sample on the ability of the second binder to bind to the first binder from the detected oxidation-reduction reaction.

93. The method of claim 92, wherein the test sample, the first binder and the second binder are each selected from the group consisting of proteins, protein fragments, recombinant proteins, recombinant protein fragments, extracellular matrix proteins, ligands, carbohydrates, steroids, hormones, drugs, drug candidates, immunoglobulins, receptors of eukaryotic, prokaryotic or viral origin, and oligonucleotides.

94. The method of claim 92, wherein the test sample and the labeled second binder are added to the immobilized first binder simultaneously.

95. The method of claim 92, wherein the labeled second binder is added to the immobilized first binder before the addition of the test sample to determine the effect of the test sample on the binding interactions between the first binder and the second binder.

96. The method of claim 92, wherein the label is an exogenous label.

97. The method of claim 96, wherein the label is an oligonucleotide.

98. The method of claim 96, wherein the label is a peptide containing amino acids capable of being oxidized in an oxidation-reduction reaction.

99. The method of claim 98, wherein the peptide label contains one or more amino acids capable of being oxidized in an oxidation-reduction reaction at approximately ≤ 0.6 V.

100. The method of claim 99, wherein the transition metal mediator is osmium^{2+}(4,4'-dimethyl-2,2'-bipyridine)$_3$.

101. The method of claim 92, wherein the nonconductive layer is the immobilized first binder.

102. The method of claim 92, wherein the nonconductive layer to which the first binder is immobilized is selected from the group consisting of streptavidin, avidin, protein A, protein G, and antibodies.

103. The method of claim 92, wherein the nonconductive layer to which the first binder is immobilized is a silane molecule covalently attached to the conductive substrate, said silane molecule further capable of forming a covalent bond with the first binder.

104. The method of claim 92, wherein the nonconductive layer to which the first binder is immobilized comprises one or more components.

105. A method of determining the presence or absence of a target protein in a test sample, said target protein having an endogenous label capable of being oxidized in an oxidation-reduction reaction, comprising:

a) providing an electrode comprising a conductive substrate modified with a non-conductive layer having an immobilized binder capable of binding the target protein and through which layer a transition metal mediator can freely move to transfer electrons to the conductive substrate;

b) contacting the immobilized binder with the test sample to form a target complex if the target protein is present in the test sample;

c) contacting the electrode, the immobilized binder and the target protein, if present, with a transition metal mediator that oxidizes the label in an

oxidation-reduction reaction between the transition metal mediator and the label, from which label there is electron transfer to the transition metal mediator resulting in regeneration of the reduced form of the transition metal mediator as part of a catalytic cycle;

d) detecting the oxidation-reduction reaction; and

e) determining the presence or absence of the target protein in the test sample from the detected oxidation-reduction reaction.

106. The method of claim 105, wherein the immobilized binder is selected from the group consisting of immunoglobulins, receptors, proteins, and oligonucleotides.

107. The method of claim 106, wherein the immobilized binder is a receptor of eukaryotic, prokaryotic or viral origin.

108. The method of claim 106, wherein the immobilized binder is an extracellular matrix protein.

109. The method of claim 105, wherein the nonconductive layer is the immobilized binder.

110. The method of claim 105, wherein the nonconductive layer to which the binder is immobilized is selected from the group consisting of streptavidin, avidin, protein A, protein G, and antibodies.

111. The method of claim 105, wherein the nonconductive layer to which the binder is immobilized is a silane molecule covalently attached to the conductive substrate, said silane molecule further capable of forming a covalent bond with the binder.

112. The method of claim 105, wherein the nonconductive layer to which the binder is immobilized comprises one or more components.

113. A labeled member of a binding pair useful for mediated catalytic electrochemistry, comprising:

a) a binder selected from the group consisting of proteins, protein fragments, recombinant proteins, recombinant protein fragments, extracellular matrix proteins, ligands, carbohydrates, steroids, hormones, drugs, drug candidates, immunoglobulins, receptors of eukaryotic, prokaryotic or viral origin, and oligonucleotides; and

b) an exogenous peptide label containing one or more modified amino acids capable of being oxidized in an oxidation-reduction reaction at potentials below those of naturally occurring amino acids.

114. The labeled member of a binding pair of claim 113, wherein the binder is an antibody.

115. The labeled member of a binding pair of claim 113, wherein there are at least two modified amino acids in the peptide label.

116. The labeled member of a binding pair of claim 113, wherein the modified amino acids in the peptide label are selected from the group consisting of derivatives of tyrosine and derivatives of tryptophan.

117. The labeled member of a binding pair of claim 116, wherein the modified amino acids in the peptide label are selected from the group consisting of 5-hydroxytryptophan; 3-aminotyrosine; and 3,4-dihydroxyphenylalanine.